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Bioavailability (the availability to biota) of organic contaminants is a critical issue for DOD installation restoration programs. Reduced availability of contaminants to biodegrading microbial populations frequently is a rate limiting problem in site cleanup of soils, groundwater, and freshwater and marine sediments. Contaminants may interact with soil and sediment particles to create complex residues which are protected from attack by microbial enzymes. Chemical solubility and sorption to surfaces of clay or colloidal organic matter may also alter the availability of contaminants to microorganisms. Mass transfer limitations (e.g., pore diffusion) may limit the rate of biodegradation and hence bioavailability. Slow leaching of contaminants from soils has been a problem in sites which have been remediated. Furthermore, physical characteristics of the soil or sediment system may limit transport of bacteria with biodegradation capabilities to the contaminant sites within the porous media. A recent report by the American Academy of Microbiology identified the "significant rate limiting effects of solubility and bioavailability" as a limitation of our current knowledge in bioremediation. Clearly, a fundamental understanding of the processes that determine bioavailability is critical to successful and cost-effective site clean-up. This workshop was developed to provide a multi-disciplinary perspective of the state of knowledge of bioavailability and establish future research needs.

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in Soils and Sediments*

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Dr. David S. Kosson
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Department of Defense Tri-Service Workshop:

Bioavailability of Organic Contaminants in Soils and Sediments

Monterey, California, April 9-12, 1995

Workshop Mission Statement

Bioavailability (the availability to biota) of organic contaminants is a critical issue for DOD installation restoration programs. Reduced availability of contaminants to biodegrading microbial populations frequently is a rate limiting problem in site cleanup of soils, groundwater, and freshwater and marine sediments. Contaminants may interact with soil and sediment particles to create complex residues which are protected from attack by microbial enzymes. Chemical solubility and sorption to surfaces of clay or colloidal organic matter may also alter the availability of contaminants to microorganisms. Mass transfer limitations (e.g., pore diffusion) may limit the rate of biodegradation and hence bioavailability. Slow leaching of contaminants from soils has been a problem in sites which have been remediated. Furthermore, physical characteristics of the soil or sediment system may limit transport of bacteria with biodegradation capabilities to the contaminant sites within the porous media. A recent report by the American Academy of Microbiology identified the "significant rate limiting effects of solubility and bioavailability" as a limitation of our current knowledge in bioremediation. Clearly, a fundamental understanding of the processes that determine bioavailability is critical to successful and cost-effective site clean-up. This workshop was developed to provide a multi-disciplinary perspective of the state of knowledge of bioavailability and establish future research needs.

Keynote Speaker:

Martin Alexander
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Bioavailability: The Neglected Frontier

Sorption to soil and sediment, presence in nonaqueous-phase liquids (NAPLs) and sequestration within environmental matrices are major determinants of the bioavailability of organic contaminants. The role of sorption in affecting bioavailability has been the subject of much research, but major questions remain in regard to its importance for compounds of environmental significance. NAPLs are widely recognized as major reservoirs of environmental pollutants, but the impact of NAPLs on bioavailability has scarcely been explored. Knowledge of the role of sequestration of pollutants on their biodegradation, bioremediation and toxicity is almost wholly lacking. Issues and problems relating to the impact of sorption, NAPLs and sequestration on bioavailability will be considered.

Session I.

**PHYSICAL/CHEMICAL DETERMINANTS OF SORBED
CONTAMINANT BIOAVAILABILITY**

Bioavailability - The Role of Chemical Speciation

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The chemical forms or species in which organic compounds occur in environmental and engineering systems plays a major role in the bioavailability of those substances. This is not only true for issues of mass transport to degradative entities (e.g., bacteria), but also for understanding what may ever be available. In this talk, I will discuss results concerning the "speciation" of hydrophobic organic compounds in sediment systems (marine beds and groundwater aquifers). Such results may help us predict the rates of biological processing for different types of organic compounds.

Bioavailability of Hydrophobic Organic Contaminants from NAPLs

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Some organic liquids derived from fuel utilization and industrial manufacturing processes comprise multi-component, hydrophobic organic compound (HOC) mixtures, such as PAHs in coal tar or creosote, or PCBs in hydraulic oils. If released to the subsurface environment, these non-aqueous phase liquids (NAPLs) can function as long-term sources of HOCs, resulting in persistent soil and water pollution problems.

The bioavailability of organic contaminants results from competing rates of diffusion, desorption, or solubilization versus the intrinsic rate of microbial biodegradation. Soil mixing of various types will likely be necessary to achieve effective soil biotreatment rates in field situations. Treatment end points need to be defined in consideration of both technical feasibility and rational soil quality criteria.

Current research is evaluating the solubilization of PAHs from coal tar, and PCB congeners from hydraulic oil. Naphthalene biodegradation is being studied in coal tar slurry bioreactors. Mass transfer and biomineralization experiments with coal tar have shown that the biotransformation of naphthalene in slurry treatment systems may be limited by either mass transfer or biokinetic phenomena, depending on the size dimension and number density for distinguishing the rate-limiting phenomena. These tests provide useful information on the maximum possible rates and endpoints of naphthalene biotransformation from coal tar.

Collaborative studies are evaluating land farming treatment of sediment contaminated with a PCB/oil mixture, and assessing the leaching of PCBs from sediment before and after land farming biotreatment. Column experiments with clean sand are underway to assess the rate of dissolution of dichloro-PCB and tetrachloro-PCB from a hydraulic oil NAPL present at residual saturation. Field test plots and laboratory column studies with PCB-contaminated sediment are investigating whether land-farming treatment may substantially reduce potentially mobile, i.e., leachable, PCB compounds or congeners.

Measuring Bioavailability of Hydrophobic Organic Compounds Using Supercritical Fluid Extraction

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Bioremediation is an attractive option for removing organic contaminants from soils or sediments. Successful application of the technology requires consideration of many microbiological and physicochemical factors. Desorption of the contaminant from the soil or sediment matrix has been identified as one of the most important physicochemical factors controlling bioavailability. Often the rate and ultimate extent of the biodegradation process is limited by the rate and extent of contaminant desorption. Desorption of contaminants from soils and sediments is often a complicated function of contaminant properties, soil characteristics and the contaminant-soil contact time. The objectives of this research are to: (i) improve the understanding of how soil characteristics and contact time affect desorption processes and (ii) to develop a supercritical fluid extraction methodology for characterizing the desorption potential and, indirectly, the bioavailability of an organic contaminant.

Soils and sediments are comprised by a complex, heterogeneous mixture of mineral and macromolecular natural organic matter (NOM) fractions. Sorption of non-polar organic compounds is primarily controlled by the NOM fraction. Previous research in our laboratory has highlighted the importance of diagenetic processes in determining the reactivity of the NOM fraction. Diagenetic processes generally act to reduce the polarity, increase the molecular weight and increase the aromaticity of NOM fractions. The conversion of humic materials to kerogen over geologic time scales represents one example of this process. Soils and sediments containing NOM subjected to extensive diagenetic alteration exhibit highly nonlinear sorption isotherms, and significantly greater sorption capacities for non-polar organic chemicals. To improve understanding of the sorption/desorption process, we have measured phenanthrene sorption energies as a function of solid-phase loading on several soils and subsoils. These results confirm the underlying heterogeneity of sorption reactions on diagenetically altered NOM. They also show promise for correlating desorption potentials of organic contaminants. Because these measurements are time-consuming in aqueous systems, an improved method was sought for determining binding energies.

A supercritical fluid extraction (SFE) method has been developed to perform these measurements. The rapid mass transfer rates and controllable solvent strength of SFE make it ideal for this application. Binding energies are characterized by extracting a sample of contaminated soil under a series of successively more stringent SFE conditions and measuring the incremental amount of contaminant extracted. Desorption isotherms of this type have been measured on several soils and subsurface materials under a variety of SFE conditions. These results are used to calculate binding energies. Aqueous desorption rates and equilibria measured for the same materials are then correlated with the binding energies. Future work will focus on making the final connection between desorption rates and contaminated soil treatability that will permit use of the SFE technique as a screening tool for contaminant availability. Implications of these data for analytical or remedial SFE applications are briefly discussed.

Effect of Biosurfactant-Enhanced Bioavailability on Biodegradation of Organic Contaminants

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Bioavailability of organic contaminants can be severely limited by low aqueous solubility and by high sorption of the contaminant. In turn, limited bioavailability of organic compounds can cause slow rates of biodegradation. Microorganisms have evolved several mechanisms to enhance bioavailability of organic compounds, one of which is production of surfactants (biosurfactants). Such surfactants have varied chemical structures and exist in either anionic or nonionic forms. The objective of this work was to examine the influence of rhamnolipid biosurfactants on the availability of organic contaminants for biodegradation. A variety of rhamnolipid structures are produced by different *Pseudomonas* sp. and a series of these structures, both anionic (monorhamnolipid acid; dirhamnolipid acid) and nonionic (monorhamnolipid methyl ester; dirhamnolipid methyl ester), were evaluated for their ability to increase the apparent solubility and biodegradation of model hydrocarbons.

Results from solubilization studies show that both biosurfactant structure and the physical state of the organic molecule (liquid or solid) influence the ability of the biosurfactant to solubilize the organic. For example, for a liquid *n*-alkane, hexadecane, apparent solubility was enhanced more in the presence of the dirhamnolipid methyl ester than the dirhamnolipid acid. This difference can be expressed in terms of a molar solubilization ratio (MSR = moles of organic compound solubilized/mole of surfactant). The MSR for hexadecane in the presence of the dirhamnolipid methyl ester was 5.2 while the MSR in the presence of the dirhamnolipid acid was 0.13, a 40 fold difference. In contrast, MSR values for octadecane, a solid-*n*-alkane, in the presence of either dirhamnolipid forms, were similar (0.74; methyl ester and 0.15; acid).

Biodegradation studies were performed using 8 *Pseudomonas* isolates. In all experiments, very low rhamnolipid concentrations were used (0.01 to 0.05mM). Results showed that in most cases, increased rates of biodegradation resulted from biosurfactant-enhanced solubilization of the model hydrocarbons studied. For the 8 isolates studied, growth on the liquid alkane, hexadecane, was stimulated from 3 to 38-fold by the dirhamnolipid methyl ester. In contrast, the dirhamnolipid acid stimulated growth more modestly for 6 isolates (1.6 to 21.7-fold) and had no effect on 2 isolates. Growth on the solid alkane, octadecane, was stimulated by the dirhamnolipid methyl ester for 7 isolates (1.2 to 4.9-fold) and inhibited for 1 isolate. The dirhamnolipid acid stimulated growth slightly for 4 isolates (1.4 to 2.7-fold), had no effect on 1 isolate, and inhibited growth of the remaining three isolates. This work demonstrates both the potential for biosurfactants in low concentration to increase bioavailability and biodegradation of slightly soluble organic contaminants, and the importance of structure to function of the biosurfactant.

Novel Application of NMR to Determine Binding of Pollutants in the Soil

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Associations between organic pollutants and soil components constitute a major factor determining the bioavailability of pollutants and hence their impact on the environment. However, the multitude of physical and chemical interactions between constituents within the terrestrial systems (covalent binding, van der Waals forces, H-bonding, ion exchange, hydrophobic bonding, ligand exchange, or entrapment in humus structure) make it difficult to predict bioavailability. It is generally accepted that covalent binding of pollutants to soil restricts their availability to organisms. Studies with ^{14}C -labeled pollutants have indicated that a covalent interaction is possible. However, direct evidence for the formation of covalent bonds can be obtained by using ^{13}C -labeled chemicals and ^{13}C -NMR spectrometry. The major advantage of this approach lies in the fact that pollutant molecules enriched with the ^{13}C isotope generate more intensive NMR signals than those resulting from the natural abundance of ^{13}C in the studied compound and soil. Changes in the location of these signals on the NMR spectra can provide not only evidence for the occurrence of covalent binding but also information on the type of bond formed between a pollutant and humic material.

Our ^{13}C NMR studies were carried out using two ^{13}C -labeled pollutants: 2,4-dichlorophenol, a degradation product of the herbicide 2,4-D, and cyprodinil, a new phenyl-pyrimidine amine fungicide manufactured by Ciba-Geigy. 2,4 Dichlorophenol, labeled either in the C-1 or in the C-2 and C-6 position, was incubated for 2 hours with equal amounts of humic acid in the presence of a peroxidase. The NMR signals generated by the ^{13}C label demonstrated bonding between the two components through carbon-carbon, ester and phenolic ester linkages. To investigate the formation of covalent bonds under more natural conditions, cyprodinil, which was labeled either in the phenyl or pyrimidyl ring, was incubated for several months with fresh soil. After exhaustive washing with methanol, humic acid was isolated by extraction with 0.5 NaOH. Humic acid was then purified by dialysis and/or silylated by the treatment with trimethylchlorosilane to facilitate the ^{13}C NMR analysis. The NMR signals generated both by the dialyzed and silylated samples indicated that covalent binding was due to transformation of ^{13}C -cyprodinil to hydroxylated derivatives and formation of oxygen bridges between the fungicide and humic acid.

Our results, especially those related to binding in the soil, confirm the great potential of using ^{13}C -labeled chemicals and ^{13}C -NMR spectrometry as an analytical technique for evaluating interactions between pollutants and soil components, particularly humic substances. Establishing the physical or chemical associations between pollutants and humic substances will provide essential data for understanding principles of bioavailability.

Session II.

**EFFECTS OF BIOLOGICAL PROCESSES ON BIOAVAILABILITY OF CONTAMINANTS
IN SOILS AND SEDIMENTS**

The Role of Biota in Controlling Bioavailability of Contaminants

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Bioavailability results from an interaction between an organism and a substrate. As such, properties of both "reactants" are critical to the eventual reaction. This review will discuss properties and actions of organisms that influence the eventual exposure of organisms to uptake or toxicity of contaminants. The discussion is divided into two general areas. First, organisms have properties that affect their own exposure to contaminants. Physical attributes, such as size, morphology and mode of encounter with various substrates (habit, food, respiratory oxidants), can dominate interphylectic comparisons of exposure. Biochemical and physiological characteristics include the nature of absorptive surfaces, changes to the chemical milieu of the substrate (e.g., digestive fluids), and nutritional status. Second, organisms may influence their environment in ways that change the overall ecosystem bioavailability. Examples include bioturbation that changes redox environments, or passage through digestive tracts. Such indirect processes may influence which segment of a biotic community is exposed to contaminants.

Microbial Determinants of Sorbed Contaminant Bioavailability

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Bacteria differ in their abilities to access and degrade organic contaminants associated with soils. The ability of some organisms to degrade sorbed, presumably surface-localized contaminants, facilitates desorption of additional contaminant residing in the interior of soil aggregates through the establishment of steep contaminant concentration gradients near the particle surface. To elucidate which attributes of bacteria were related to their efficiency of sorbed contaminant utilization, we examined the surface chemical, physiological, and behavioral characteristics of two organisms which differ in their ability to degrade soil-sorbed naphthalene. Surface chemical studies included measurements of whole cell electrophoretic mobilities following growth on naphthalene or alternate carbon sources, and cell surface hydrophobicity assays using hydrophobic interaction chromatography with radiolabeled cells. Physiological characterization of cells included determination of enzyme kinetic parameters and enzyme maintenance and induction kinetics, and the influences of organic and inorganic soil components on degradative activities in the absence of sorption. We also examined the attachment, motility, and chemotactic behaviors of the organisms using viable counting and microscopic methods, and a modified capillary assay.

A peritrichously flagellated *Alcaligenes* (strain NP-Alk) grew in minimal medium with naphthalene as the sole carbon and energy source by colonizing naphthalene particles. This organism showed positive chemotaxis toward naphthalene with saturation-type response as naphthalene concentration increased. Strain NP-Alk, typical of oligotrophic bacteria, showed a low K_m and a low V_{max} for naphthalene and grew very efficiently with naphthalene as a substrate. Despite these seemingly favorable characteristics, strain NP-Alk was unable to utilize sorbed naphthalene or to facilitate its desorption from soil. The organism, even during growth on naphthalene, had a relatively hydrophilic cell surface. It also showed extensive and irreversible attachment to soil particles which limited its ability to deplete naphthalene at sites on the soil surface. Avid attachment of this strain was also thought to occlude a portion of the cell surface and limit its potential for naphthalene uptake from the aqueous phase.

A monotrichously flagellated *Pseudomonas putida* (strain 17484) also grew in minimal medium with naphthalene as the sole carbon and energy source, but did not attach to the naphthalene particles. This organism was also positively chemotactic toward naphthalene, but showed a peak response at intermediate concentrations. When grown on naphthalene, strain 17484 was relatively hydrophobic and less electrophoretically mobile than strain NP-Alk. This organism attached in a reversible manner to soil and accumulated in high numbers in chemotaxis capillaries loaded with soil colloids. Sorbed naphthalene was directly available to strain 17484 and complete desorption of bound naphthalene was facilitated by this organism. It is believed that the reversible attachment of this strain to soil, its hydrophobic cell surface, and its concentration-dependent chemotactic behavior allowed multiple interactions of each cell with the sorbent, thus creating numerous zones of naphthalene depletion at the soil surface. Desorption of naphthalene bound in the interior of soil particles was facilitated by the concentration gradients established between the sorbent interior and the sorbent surface, rather than between the sorbent and the aqueous phase.

Obstacles to Understanding the Sorptive and Microbial Behavior of Organic Contaminants in Field Sites: Soil Spatial Heterogeneity and Artifacts Caused by Both Microbial Metabolic Activity Assays and Sterilization of Sediments via Gamma Irradiation

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Attempts to assess the behavior of organic contaminant compounds in field sites frequently rely upon the results of laboratory assays designed to measure the chemical and microbiological characteristics of field-derived samples. Not only are concentrations of organic contaminants routinely determined, but sorption/desorption tests and microbial metabolism assays are also commonly implemented. The data generated may serve as a basis for site remediation projects. In attempting to understand the persistence of naphthalene and phenanthrene in a coal-tar contaminated field site, we have: (1) used gamma irradiation to sterilize sediments for examining how the duration of phenanthrene-sediment contact time (aging) influences subsequent biodegradation of phenanthrene; (2) used solid phase extraction procedures at the field site, in combination with GC/MS analyses, to determine a key intermediary metabolite, 1,2-dihydroxy 1,2-dihydronaphthalene, in site-derived water; (3) conducted anaerobic sediment incubations later amended with air to implicate oxygen as a limiting physiological factor for microbial metabolism of naphthalene; and (4) used the results of O₂-limitation laboratory tests to guide *in-situ* sediment manipulations in which small, randomly arranged, replicated field cores were amended with hydrogen peroxide in order to encourage aerobic naphthalene metabolism.

The above four experimental procedures fell short of delivering the desired information on the behavior of organic contaminants. Gamma-irradiation of the sediment (necessary to eliminate phenanthrene metabolism during the aging period that preceded introduction of biodegrading microorganisms) changed the K_d of the sediment and in some cases completely inhibited phenanthrene biodegradation. Processing required for solid-phase extraction of site-derived waters (designed to rapidly trap and isolate intermediary metabolites indicative of *in-situ* naphthalene metabolism) may have generated artificially high concentration of the 1,2-dihydroxy-1,2-dihydronaphthalene intermediary metabolite. Oxygen-limited naphthalene metabolism was readily demonstrated in laboratory-incubated sediment slurries; however, despite delivery of 250 ppm peroxide to *in-situ* soil cores, the heterogeneous distribution of naphthalene in the field prevented demonstrating that peroxide treatment stimulated naphthalene loss in amended versus unamended cores.

The Biodegradability of Fuel Components in Soil and Sediments: Mineralogical Effects on Bioavailability

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Contaminated soils and sediments can pose risks to aquatic ecosystems and to human health through food chain bioaccumulation and biomagnification. A significant proportion of this impact is from fuel components. Petroleum fuels are composed of aliphatic and aromatic organic compounds, each of which differs in its reactivity, solubility, toxicity, volatility, mineral surface affinity, and biodegradability. The biogeochemical behavior of each component in such complex mixtures may be different than it would be in isolation from other organic constituents. Thus, extreme caution should be used in setting up and interpreting experiments to determine biodegradability, risk, and bioavailability of anthropogenic organic components in soil and sediment systems.

Fuel components in soils and sediments are generally measured by "standard" methods such as GC-FID to determine total petroleum hydrocarbons (TPH) or by infrared spectroscopy (IR) to determine total number of C-H bonds. However, both these methods are subject to interference from natural organics and can obscure the significantly less abundant, but more toxic, polycyclic aromatic hydrocarbons (PAHs). PAHs can be selectively monitored by GC-MS or by radiolabeling. A number of experiments have been carried out to determine the biodegradability of fuel components (TPH, *n*-alkanes and PAHs) in complex mixtures.

Experiments were carried out using whole fuel mixtures in both simple and complex marine sediments under aerobic conditions, using indigenous microorganisms from soils and sediments from San Diego, California. TPH, as well as specific *n*-alkane biodegradation was slow, often incomplete, and very sensitive to sediment composition, in spite of the fact that *n*-alkanes are generally believed to be the most easily mineralized fuel components. On the other hand, PAHs, which are generally considered more resistant to mineralization, degraded rapidly and more completely regardless of sediment composition. For instance, in preliminary experiments carried out to determine the effects of substrate mineralogy on the biodegradability of fuel components, samples of quartz sand (Fisher Sea Sand) and illite clay (API#35) were spiked with marine diesel fuel (DFM), aged, slurried, and inoculated with microorganisms, and then concentrations of fuel components were monitored over the time. While there was clear chromatographic evidence of TPH biodegradation on the sands, illite samples showed no evidence of biogenic loss of aliphatic components. PAHs, on the other hand, degraded equally well on both substrates, and, in both cases, degraded to a much greater extent than did TPH. These preliminary results have been confirmed using additional substrates (montmorillonite, humic material and sea water) and radiolabelled compounds. Thus, even when standard methods would suggest ineffective biodegradation of fuel on some substrates, careful examination of the system showed that the more toxic and carcinogenic components were substantially mineralized. Since the ultimate goal of bioremediation is the reduction of toxic effects, PAH biodegradation may be more critical than a reduction in TPH levels. These results suggest that soil and sediment composition can affect biodegradability, perhaps via controls on bioavailability.

Session III.

**MATHEMATICAL MODELING OF ORGANIC CONTAMINANT BIOAVAILABILITY
IN SOILS AND SEDIMENTS**

Volatile Organic Contaminant Release and Transport in Unsaturated Soils

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Biodegradation of organic contaminants in soils and sediments requires the confluence of the contaminant of interest and the organism with appropriate degradative capability in a porous media. For soils, the contaminant most frequently must be in an aqueous phase at a concentration greater than the minimum concentration which can be utilized by the bacteria. This aqueous phase, in turn, must be present at a location in the soil pore structure which is accessible to the organisms without size exclusion. Concurrent biodegradation and contaminant desorption may result in biodegradation rates which are limited either by the intrinsic biodegradation kinetics or the rate of contaminant mass transfer within the system. Thus, critical factors which must be quantified include contaminant partitioning equilibrium and mass transfer rates, and microbial transport and intrinsic biodegradation kinetics. Quantitatively, these factors are a function of soil, contaminant and microbial characteristics. For sediments, these factors are further complicated by the presence of larger organisms which can achieve bulk particulate transport between different electron acceptor, substrate and redox zones. Particulate transport through ingestion and excretion also can transform the chemical, physical and bacterial composition of particle aggregates.

This presentation provides an overview of factors which must be quantitatively described to mathematically model limits of biodegradation within soil and sediment systems. Subsequently, the presentation focuses on approaches for volatile organic contaminant transport in unsaturated soils. Contaminant equilibrium partitioning is described through consideration of multi-phase equilibrium amongst solid, aqueous, organic liquid and vapor phases. Partitioning amongst fluid phases is considered linear while partitioning between fluid and solid phases is considered to follow a BET isotherm model. Phase distribution is described based on overall system composition and soil physical properties. Contaminant mass transfer rates under diffusion controlled conditions are described in both liquid and vapor phases through consideration of parallel and serial resistances based on system composition and physical characteristics.

Leaching Characteristics of Organic Solutes from NAPL Mixtures in Contaminated Media

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Frequently, contaminants within the source zone of contamination are components of NAPL mixtures confined within the porous media. An understanding of the factors which govern the release of contaminants from contaminated media containing NAPL mixtures is essential in assessing the bioavailability of contaminants from such sources. In this paper, a mathematical model will be presented to describe the short-term and long-term leaching behavior of organic contaminants from a source zone containing a NAPL mixture. This model incorporates both equilibrium partitioning of the contaminants and potential mass transfer limitations - incorporating mass transfer limitations is one way to account for the asymptotic leachate concentrations that are observed in long-term leaching column studies. Model predictions are compared with the results from soil column experiments for BTX (benzene, toluene, xylene) and selected PNA components (naphthalene, phenanthrene, pyrene, and benzo(a)pyrene) present in a model NAPL (tridecane) source confined within a short zone near the column inlet.

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The Kinetics of Organic Ligand Biodegradation in the Presence of Common Sesquioxide Grain Coatings

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Radionuclides and metals can be mobilized by chelating agents typically present in the low-level radioactive liquid wastes that are disposed in shallow land trenches. The persistence of the organic chelating agent in the subsurface environment is a critical control on the impact that it may have on solubilization and transport of the complexed nuclide. Low-molecular-weight organic compounds are susceptible to uptake by bacteria, yet the biodegradation rates of nuclide-ligand complexes in a mixture of solids (soil minerals) and liquid (groundwater) are poorly known. We investigated the rate of citrate uptake in the presence of cobalt by a mixed bacterial community under experimental conditions designed to explore the impacts of citrate concentration, temperature, and chemistry of mineral surfaces. A dilute solution with a typical sandy-aquifer groundwater composition was amended with equimolar amounts of cobalt and citrate ranging from 4 to 320 μM . These solutions were combined in a flask with four different sand treatments: uncoated quartz sand, Fe-coated quartz sand, Mn-coated quartz sand, and no sand. Experiments were run at 15°C and 25°C. Mineralization (net $^{14}\text{CO}_2$ produced) and assimilation (^{14}C retained on 0.2 μm filter) were quantified. Initial rates of citrate mineralization and of assimilation were lower at lower temperatures, and the effect of temperature was greater for the no-sand and the uncoated sand treatments in contrast to the Fe- or Mn-coated sand treatments. Yet, the fraction of total carbon processed that was assimilated into cells was greater at 15°C than at 25°C. At a single temperature, similar results were seen for the no sand, uncoated sand, and Mn-coated sand treatments. In the presence of Fe-coated sand, a greater fraction of carbon was assimilated by the mixed culture at both temperatures. The van Slyke equation, based on a model of sequential irreversible reactions, was fit to the results of the heterotrophic uptake experiments. The estimated value of the van Slyke constant indicates that the overall rate of citrate uptake was limited by mass-transfer constraints, and the mass-transfer limitation was greater at lower temperature and for the no-sand and uncoated sand treatments. It appears that the sorption of citrate to the sand surface (greatest sorption on Fe-coated sand, less on Mn-coated sand, much less on uncoated sand, and none in no-sand treatment) did not limit the rate of uptake. Further, complexation with cobalt altered neither the rate of citrate degradation nor the rate of bacterial growth. We conclude that the rate of uptake was limited by transmembrane citrate transport. The bacteria did derive some benefit from being associated with the solid sand surface, and it was not directly related to extent of citrate sorption. Rather, we believe a local elevated concentration of adsorbed nutrients, such as PO_4^{3-} or NO_3^- , was available to the adsorbed bacteria that allowed a more efficient processing of carbon into biomass. Sesquioxide coatings enhance the sorptive properties of the mineral grains, and bacteria derive benefit from being associated with the solid sand surface.

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Kinetics of Biodegradation of Sorbed Chemicals: Approaches in Defined Systems and Soils

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Sorbed chemicals present one of our most challenging environmental problems in the bioremediation of contaminated soils and sediments. Our understanding of the biodegradation of sorbed chemicals is confounded by the fact that the term "sorption" is often used to describe different interactions between pollutants and the soil matrix. These interactions include different mechanisms of adsorption, diffusion, bound residue formation and "aging" of residues.

To help interpret the effects of sorption/desorption and diffusion on the metabolism of organic chemicals, a simulation model was developed to describe biodegradation in the presence of aggregates. The model assumes diffusion is described by Fick's second law, sorption by a linear isotherm, biodegradation by Monod kinetics, only the solution phase of the chemical is used, microorganisms are excluded from aggregates, and sorption is instantaneous once the chemical diffuses to the sorption site. Using physical and biological measurements from experimental systems as input parameters, simulations were similar to measured data describing biodegradation of phenol by a pure culture of *Pseudomonas* sp. in the presence of clay aggregates, as well as in other well-defined systems. Sensitivity analyses of biodegradation over time were performed to determine the dependence of a chemical's "apparent" half-life on soil physical and chemical properties. Different kinetic expressions describe biodegradation depending on whether the reaction is controlled by mass transfer or the biological rate, and whether the chemical starts out as sorbed or not sorbed.

Model predictions and measured data were compared for several experiments conducted in soil or soil slurries. Model simulations showed similar trends as experimental data depicting biodegradation of low concentration of phenanthrene was measured in six soils with organic carbon (OC) contents ranging from 3.6 to 9.5%. The rates of biodegradation varied by soil, with the highest rate observed in the soil with the highest OC content. Measured K_d values for phenanthrene in the soils varied by a factor of 10, whereas K_{OC} varied by a factor of two. Linear regression analysis showed no relationship between the biodegradation rate and either K_{OC} or K_d .

It becomes increasingly difficult to extract chemicals the longer they have been in contact with soil. The decreased extractability, called aging, is associated with a decrease in the bioavailability and degradability of the chemicals. The rate of biodegradation of phenanthrene incubated in sterile soil under unsaturated conditions was substantially reduced relative to the rate in freshly amended soil, regardless of whether incubations were done under saturated conditions or in soil suspensions.

In conclusion, many pollutants assumed to be sorbed to soil have may have irreversible or partially reversible interactions with soil, or their availability to microorganisms may be limited by diffusion rather than purely desorption. Theory developed for simple sorption may not be applicable to many bioremediation conditions. Research questions to explore include: i) what different mechanisms are involved in pollutant-soil interactions and what is their impact on bioavailability, ii) what is the spatial distribution of sorbed pollutants and microbial populations in soil, iii) what specific properties of microorganisms (e.g., surfactant production, ability to attach), enhance *in-situ* biodegradation rates, and, (iv) what physical *in-situ* treatment of soil increase bioavailability without adversely affecting microbial populations.

A Natural Gradient Tracer Experiment in a Heterogeneous Aquifer with Measured *In-Situ* Hydrocarbon Biodegradation Rates

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Contamination of groundwater systems with hydrocarbon chemicals is an increasingly important environmental problem. A knowledge of the groundwater fate and transport of these chemicals is necessary to better understand long-term environmental consequences and to develop effective remedial actions. The current knowledge base is limited to homogeneous systems, and by degradation rates calculated from contaminated sites with the inherent limits on quantification due to source uncertainty.

To better understand contaminant fate and transport in heterogeneous systems, a natural gradient solute transport experiment was conducted in an uncontaminated heterogeneous aquifer at Columbus AFB, MS, USA. Tritiated water served as the conservative tracer, and benzene, p-xylene, ¹⁴C-labeled p-xylene, naphthalene, and o-dichlorobenzene were the non-conservative tracers. Plume movement was monitored over 440 days through a monitoring network consisting of over 300 multilevel sampling wells. Organic chemical concentrations were determined by gas chromatography with flame ionization detection. The resulting time, distance, and concentration data were girded and integrated. Moments analyses were then conducted on the resulting data.

Mass balances on the tritiated water were 100%, whereas the hydrocarbons all showed mass loss. The plumes were highly asymmetric and exhibited a three dimensional character. Hydrocarbon losses were greater than loss of the o-dichlorobenzene. Biodegradation was inferred by dissolved oxygen depression and demonstration of dissolved ¹⁴CO₂ measured in water samples from the test site. *In-situ* biodegradation rates were calculated for a model system and the whole field.

To fully describe fate and transport in heterogeneous systems it is necessary to obtain three dimensional data through multilevel sampling wells. Three dimensional solute transport models that include biodegradation may then be used to try to simulate and predict transport. Natural biodegradation of hydrocarbons appears to be a significant mass loss mechanism in aerobic aquifers. The data base from this experiment is available for use in testing solute transport models. Natural attenuation may be a viable treatment alternative for hydrocarbons at some field sites.

Biostabilization of PCB Contaminated Soils/Sludges

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The aerobic biodegradation of PCBs is generally limited to the lower chlorinated PCB homologs, including mono-through tetrachlorobiphenyls with some biodegradation of pentachlorobiphenyls. In addition, the total extent of PCB biodegradation that can be achieved under aerobic conditions is generally between 50 - 85% destruction as demonstrated in both lab and field studies. In light of these limitations, the question can then be posed, is PCB biodegradation a viable remediation alternative? This paper presents a conceptual approach for evaluating this limited range and extent of PCB congener degradation, in terms of bioavailability and risk analysis. Experiments have shown that at 50% total PCB biodegradation extensive degradation of the lower chlorinated homologs results, which in turn can result in a substantial decrease in the leachable fraction of PCBs. Additionally, studies have been conducted to assess the biodegradation of water-soluble PCBs in the low parts per billion range, and these have demonstrated that virtually all of the water-soluble PCBs can be biodegraded. This observation supports the hypothesis that a substantial limiting factor in the biodegradation of higher chlorinated PCB congeners is their poor bioavailability. When these observations are combined, one can present a concept of biodegrading the lower chlorinated homologs to decrease subsequent migration of the residual PCB fraction and in addition, a model can be proposed for a significant rate of degradation for any subsequently solubilized PCBs. This concept is presented as "biostabilization."

POSTER SESSION

Enhanced Bioavailability of Petroleum Hydrocarbons by Biosurfactants

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Glycolipid biosurfactants are produced by *Rhodococcus* sp. H13-A to enhance substrate solubility and promote bioavailability for degradation. This work will build on an ongoing initiative to evaluate the performance of biosurfactants in the remediation of petroleum hydrocarbons in contaminated aqueous and sediment environments. The work will proceed in three stages. The initial stage will involve production and characterization of extracellular biosurfactants by H13-A when grown on minimal salts media with hexadecane as the carbon source. The second stage will evaluate the performance of the biosurfactants in enhancing the aqueous solubility of weathered West Texas Crude. The final stage is to study the biodegradation rates of various components of petroleum hydrocarbons, and also the toxicity of the surfactant-aqueous mixtures, before and after biodegradation. Total petroleum hydrocarbons (TPH) analysis and gas chromatographic/mass spectrophotometric (GC/MS) analysis of the water-soluble fraction (WSF) will be used to determine the changes in concentrations. The toxicity of the biosurfactant aqueous petroleum mixtures will be monitored using Microtox®. Observed results include:

- Enhanced solubilization of petroleum hydrocarbons due to the surface-active biosurfactants,
- Increased degradation of petroleum hydrocarbons, and
- Initial increase in levels of toxicity due to increased solubilization of the polycyclic aromatic hydrocarbons (PAHs), but subsequent decrease due to enhanced biodegradation.

Initial results indicated production of the biosurfactants by *Rhodococcus* sp. H13-A during the stationary growth stage. Biosurfactants lowered the surface tension from 72 to ~30 dynes/cm and interfacial tension to below 5 dynes/cm, resulting in enhanced solubilization of the petroleum hydrocarbons in the aqueous phase. This increased the bioavailability of the otherwise insoluble PAHs to microorganisms for biodegradation. The two- and three-ring aromatic compounds showed a substantial increase in their aqueous phase concentrations in the presence of biosurfactants. The research is an on-going endeavor to study the effects of enhanced solubilization on the biodegradation rates of various classes of PAHs. The toxicity effects due to enhanced solubilization of the polycyclic aromatic hydrocarbons were observed. Future studies of toxicity of the biosurfactants, and the surfactant-aqueous mixtures after biodegradation will also be examined.

**Enhancing Bioavailability through Pneumatic Fracturing for the
In-Situ Cleanup of Subsurface BTX Contamination**

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A field study was conducted under the Emerging Technologies SITE Program of the U.S. Environmental Protection Agency, to evaluate the integration of two remediation technologies, pneumatic fracturing and bioremediation, for the cleanup of BTX contamination in an unsaturated, subsurface soil at an oil refinery. Initial site characterization indicated BTX levels up to 1500 mg/kg soil, in the presence of other hydrocarbons. Pneumatic fracturing with high-pressure air served to increase the permeability and mass transfer rates at the site, as well as to provide a means for the introduction of soil amendments into the subsurface. After fracturing, aqueous N and P amendments for stimulating and extending *in-situ* microbial activity were injected through the pneumatic system. A total of five amendment injections were made over a 12-month period, as calcium ammonium nitrate in a phosphate buffer solution, to provide both an alternate electron acceptor (nitrate), as well as nutrient supplementation (ammonia and phosphate) for increasing biodegradation rates. Routine sampling and analysis of soil gases from monitoring wells were used to assess activity in the subsurface. Results after one year of operation indicated a 79% reduction in soil-phase BTX concentrations. Of these losses, over 85% was attributable to biodegradation processes.

Physical/Chemical Determinants of Sorbed Contaminant Bioavailability

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The availability of soil or sediment bound organic contaminants to pollutant-degrading bacteria may be influenced by a variety of physical/chemical factors which may in turn limit the effectiveness of bioremediation technologies. To assess the bioavailability of soil-sorbed organic contaminants, we have developed a kinetic method which compares the rate and extent of contaminant mineralization by bacterial species in soil-free and soil-containing systems. Although several previous studies have concluded that sorbed contaminants are unavailable to biodegradative bacteria, we found differential bioavailability of soil-sorbed naphthalene to two bacterial species. In one case, sorption limited the rate and extent of naphthalene mineralization in accordance with the values predicted, assuming only aqueous phase naphthalene was available, similar to other previous studies. However, for a second species, both the rates and extents of naphthalene mineralization exceeded predicted values indicating direct access to the pool of sorbed naphthalene which may be localized near the surface of soil organic matter. It was postulated that utilization of this material may in turn promote the desorption of naphthalene located more deeply within the clay aggregate by establishing a steep concentration gradient between the interior and exterior of the aggregate. In addition to revealing these species-specific differences, our kinetic approach has been used to isolate several physical/chemical factors that influence the bioavailability of sorbed contaminants. Among these are the organic matter content of the sorbent (e.g., soil), the contact time between the contaminant and soil (aging), the chemical composition of the sorbent phase, the particle size of sorbent, the contaminant desorption rate, and sorption mechanisms. For bacteria accessing sorbed contaminants, higher soil organic matter content appears to manifest relatively lower bioavailability. Likewise for such bacteria, contaminant aging has been observed to decrease the bioavailability of sorbed chemicals. For bacteria unable to utilize sorbed substrate, contaminants sorbed to natural soil organic matter have slower desorption kinetics and hence are less available than when sorbed to synthetic organoclays complexes which manifest faster desorption kinetics. Likewise, desorption rate-dependent mineralization of sorbed naphthalene was observed for larger (0.25 to 1mm) organoclay aggregates, but not for smaller (<0.25 mm) aggregates which display ~10 fold faster desorption kinetics. Finally, sorption to the synthetic sorbents XAD-2 and granular activated carbon greatly reduced the bioavailability of naphthalene, as compared to sorption to natural soil organic matter, indicating important effects of sorption mechanisms on bioavailability.

Potential Role of Bioemulsifier Production by TNT-Degrading Microorganisms in Biotreatment of Explosives-Contaminated Soils

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The objective of this research was to develop a better understanding of the microbial requirements necessary for effective bioslurry and *in-situ* biotreatment systems for explosives. Soil slurries and isolates were incubated with radiolabeled TNT to assess mineralization capability, while degradation products were determined by HPLC/GC Mass Spectrometry. Microbial isolates from soil showed very weak mineralization when cultured alone (1 to 3 percent in 14 days). Furthermore, when isolates were cultured individually, the cells exhibited clumping typical of toxic by-product accumulation in microbial cells. One of the isolates, a *Pseudomonas aeruginosa*, consistently showed elevated mineralization activity (4 to 5 percent in 14 days) when paired singly with certain other bacteria. Another isolate, a *Pseudomonas nitroreducens*, supported elevated mineralization activity (3.5 to 5.5 percent in 14 days) when paired with a minimum of two other isolates. When examined for their ability to produce surfactants and/or bioemulsifiers with the use of selective media and fractionalization procedures, these two microorganisms were the only isolates exhibiting bioemulsifying activity. This capability is discussed in terms of the sorption properties of soil and the demonstrated ability of surfactant addition to enhance biological removal of TNT in bioslurry and *in-situ* biotreatment systems, possibly by accelerating movement of TNT into the solution phase.

Investigation of Enzymatic Alterations of 2,4-Dichlorophenol Using ^{13}C NMR in Combination with Site-Specific ^{13}C -Labeling: An Important Step in Understanding the Environmental Fate of this Pollutant

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The enzymatic degradation of 2,4-dichlorophenol (2,4-DCP), a major pollutant derived from herbicides, has traditionally been examined because of the potential for mitigating its toxicity by coupling to sedimentary organic matter and enzymatic mineralization. The effectiveness of such mitigation strategies is dependent upon knowledge of the various degradation intermediates which have traditionally been examined by gas chromatographic techniques (GC). Unfortunately, many of the degradation products and mechanisms are unknown because the products are presumably too polar to be analyzed by such methods. This study offers a new method for the examination of degradation products, one which involves ^{13}C NMR.

The main focus of this research is to examine the enzymatic alteration of 2,4-DCP using ^{13}C -labeled 2,4-DCP and ^{13}C NMR. The ^{13}C -labels serve as a flag to monitor chemical changes at or near the labeled sites in 2,4-DCP. Examining enzymatic alteration reactions of 2,4-DCP in this manner allows detection of all reaction products that can form. In addition, the formation of covalent bonds between 2,4-DCP and macromolecular compounds, such as humic materials, can be monitored. Because this analytical technique can monitor and characterize the covalent interactions of pollutant molecules with macromolecular complexes and also because it can identify compounds that are not GC amenable, NMR in combination with site-specific labeling is highly useful for biodegradation studies.

Using this labeling technique, it has been demonstrated that the reaction products of 2,4-DCP, in the presence of isolated enzymes and humic substances (Minnesota peat humic acid), consists of newly formed covalent bonds, many of which can be assigned to specific coupling products between the humic substances and 2,4-DCP. The results also indicated that the aromatic ring structure had remained intact during the enzymatic alteration reactions.

Extending this procedure one step further, the alteration of 2,4-DCP in the presence of ligninases, exoenzymes produced by a growing culture of *Phanerochaete chrysosporium* (white-rot fungi), was examined in the presence and absence of natural macromolecular organic matter from Suwannee River. White-rot fungi produces a non-specific exoenzyme that is capable of degrading lignin and a variety of aromatic compounds. The ^{13}C NMR results have shown that 2,4-DCP readily reacts, causing the aromatic ring to be quickly ruptured and resulting in the formation of several hydrophilic products. ^{13}C NMR data also has shown that these products contain alkene functional groups. Few of these products are amenable to GC/MS characterization. ^{13}C NMR spectra of NaOH extractions of the fungal biomass show that some of the labeled carbons are associated with the biomass, possibly as an acetyl which then degrades to an olefin-type compound.

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The Role of Sand Grain Coatings and Cell-Surface Properties in Determining Bacterial Attachment to Aquifer Solids

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The ability of bacteria to move to contaminated zones in the saturated subsurface is dictated by the attachment and detachment of the cells to the porous media grains. Aquifer solids differ in their "affinity" for microbial cells, and that affinity is modified by the surface properties of the microbial cells. Because of the like charge characteristics between bacterial cell surfaces and quartz surfaces, aquifers made up of quartz sand would not be expected to retard cell mobility in advecting ground water. The ubiquitous coatings present on mineral grains (Fe, Al, and Mn oxides, and organic matter) may offer a highly reactive surface to which bacteria readily attach. When bacterial cells were passed through a column containing synthetically prepared Fe(III)-oxyhydroxide-coated quartz grains, all bacterial cells were retained; whereas, columns prepared with clean, uncoated quartz sand eluted approximately 10-90% of the added cells. Mixtures of clean and Fe(III)-coated sand retained cells in proportion to the amount of Fe(III) in the porous medium. The combined effect was interpreted as extensive, irreversible sorption to Fe(III)-coated grains and reversible, linear sorption to clean quartz grains. Organic coatings modify the effects of the underlying inorganic coatings in a complex way that includes modification of both the electrostatic and hydrophobic properties of the solid surface. The balance between attachment and detachment may be shifted by cell-surface properties such as hydrophobicity or surface charge. When a variety of strains of widely differing hydrophobicities were passed through packed sand columns in low-ionic strength carrier solution, the variable extent of retention of cells did not reflect the wide range of hydrophobicities, and the correlation between retention and hydrophobicity was weak. In dilute solutions, the primary factor in determining extent of bacterial attachment is the electrostatic attraction between the mineral surface and the bacterial cell. At higher ionic strengths, the electrical double layer collapses making the hydrophobic effect the dominant feature of the solid-cell interaction. By careful determination of the nature of aquifer solids and groundwaters and by selection of bacterial strains for favorable cell-surface properties, it may be possible to facilitate the movement of bacteria into contaminant plumes, thus making the contaminant available to non-indigenous organisms for biodegradation.

Enhancing the Bioavailability of Low-Solubility Hydrocarbons Using Surfactants above Their Critical Micelle Concentration

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Low-solubility polycyclic aromatic hydrocarbons such as phenanthrene are easily biodegradable when they are dissolved in water, but due to sorption onto soil and/or their presence in a non-aqueous phase, their bioavailability is greatly reduced. In an aqueous environment containing surfactants above their critical micelle concentration, hydrophobic contaminant will partitioning into the hydrophobic core of the micelles. This partitioning enhances the apparent solubility of these hydrocarbons and therefore also their desorption from soils. Conceivably, in the absence of any inhibitory effects, such surfactants may therefore enhance the biodegradation of the hydrocarbon if the hydrocarbon that has partitioned into the micellar phase is bioavailable. Through a set of screening experiments, a group of non-ionic surfactants was identified which did not inhibit the bacterial degradation of the phenanthrene. A mathematical model was formulated to describe the interaction of the biomass-contaminant-water surfactant system. The model considers the surfactant effects on the overall solubility of phenanthrene through the partitioning of the phenanthrene into the micelle, and in its development it was assumed that the surfactant does not affect the biochemical characteristics of the biomass. These assumptions were verified experimentally with a series of batch-type experiments. An effective bioavailable concentration of phenanthrene was defined as the mass of phenanthrene that is dissolved in the water phase plus a fraction of the mass associated to the micellar phase. The model simulates experimental data for the biodegradation of phenanthrene in the presence of surfactants well, indicating that a fraction of the phenanthrene in the micelle phase can be degraded simultaneously with the phenanthrene in the aqueous phase. The bioavailable fraction of phenanthrene associated to the micellar phase was found to be a function of surfactant type, surfactant concentration, and biomass concentration. An interesting finding was that the bioavailable fraction associated with the micellar phase decreases as the micellar number increases. Experiments and mathematical simulations were also conducted for the biodegradation of phenanthrene in the presence of surfactants and soil. The sorption loss of surfactant onto soil was found to be significant, and capable of undermining the enhancement of the bioavailability, since the surfactant sorbed to soil also enhances the sorption of phenanthrene onto soils.

Microscale Phenanthrene Diffusion in Unsaturated Soil

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Process-based biodegradation models require pollutant concentrations in the soil solution as inputs. In batch studies, an average soil solution concentration is often assumed to represent the initial pollutant concentration encountered by soil microbes. Uneven distribution of a strongly sorbing pollutant could create high local pollutant concentrations preventing accurate modeling. Biodegradation kinetics were used as a probe to measure the homogeneity of phenanthrene distribution in spiked soils. Phenanthrene biodegradation kinetics in mixtures of two soils with different sorption properties did not depend on which soil initially received the phenanthrene, but rather on the overall composition of the mixture. Calculations assuming equilibrium partitioning of phenanthrene between solid, air, and water soil components and utilizing an average effective diffusion coefficient suggest phenanthrene might diffuse 100's of microns in 24 hours. This small movement may be enough to average the chemical's distribution across the soil components before significant biodegradation occurs. The equilibrium partitioning assumption probably underestimates initial transport by diffusion since sorption kinetics are thought to be diffusion controlled. In addition, micron level diffusion distances may not be large enough to ignore microscale soil heterogeneity.

***In-Situ* Bioemulsifier Cleaning of Oil:Water Separators (OWS)**

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To operate ships in compliance with state, federal and international laws applying to oily waste discharge, parallel plate oil:water separators (OWS) are used to remove petroleum products from shipboard oily bilge water. Separation of oil and water is dependent on laminar flow of oily bilge water through parallel, corrugated polypropylene plates. Periodic removal and hand cleaning of the polypropylene plates ensures the separator's effluent will comply with the oily waste discharge regulations. Hand cleaning of the OWS is labor intensive and can expose personnel to toxic/hazardous sludge within the tank. Synthetic surfacants and emulsifiers are unsuitable because they produce excessive foam and repeated rinsing is necessary to remove them and restore laminar flow characteristics to the OWS. Bioemulsifiers and biosurfactants have an advantage over synthetic emulsifiers and synthetic surfactants because the biological molecules can be destroyed by the application of heat, chemicals or enzymes (i.e. emulsanases). An *in-situ* bioemulsifier cleaning process was developed for removal of the oily sludge from the OWS. This process is an ecologically sound method of cleaning OWSs that would not expose personnel to hazardous sludge and hydrogen sulfide generated by anaerobic bacteria within the tank.

Bacterial emulsifiers and biosurfactants have been used by other investigators to clean crude oil storage tanks and in oil recovery processes. Bacterial emulsifiers solubilize petroleum products, function in high salinity conditions and over a wide pH range. Laboratory testing was done to compare the effectiveness of bioemulsifiers and biosurfactants in the removal of oily sludge from polypropylene plates. Laboratory studies on optimization of emulsification were done. Selected bioemulsifiers and bioemulsifiers with biosurfactants were tested in a 1 gpm OWS. The bioemulsifiers and biosurfactants tested were chemically unreactive toward the components of the separator. The OWS has parallel plates about 5 mm apart to maximize laminar flow and minimize turbulent flow. Various methods for increasing mixing of microbial emulsifiers and microbial surfactants with the oily sludge in the 1 gpm OWS were tested. Air sparging generated sufficient agitation to mix the bioemulsifier with the oily sludge on the parallel plates in the separator. After the laboratory testing, shipboard evaluations will be done. A hazardous materials assessment for potential harmful health effects was conducted by the Navy Environmental Health Center (NEHC) in Norfolk, VA on a commercially available bioemulsifier selected from our testing. This bioemulsifier product was recommended by NEHC as safe for shipboard application when used as directed for the intended purpose following standard operating procedures (SOP). The emulsified oil waste generated from the *in-situ* cleaning process would be handled on-shore in an environmentally safe way (i.e., bioremediation or incineration). The Navy is currently building a facility at Craney Island, VA for treating oily bilgewater, off-spec fuels, etc. using biodegradation, followed by 4-5 h of ozonation to destroy recalcitrant hydrocarbons present in the water after the biodegradation process.

Relationships between Desorption and Mineralization of Adsorbed Organic Compounds in a Sewage Sludge-Amended Agricultural Soil.

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Since organic chemicals that are distributed or spilled onto agricultural soils may first interact with components of the soil matrix prior to biodegradation, we wanted to investigate how these interactions affected subsequent microbial mineralization kinetics. Five ¹⁴C-chemicals (anionic, cationic and nonionic surfactants) were aseptically sorbed to montmorillonite, kaolinite, illite, sand, humic acid and fulvic acid. The complexes were added to a sludge-amended agricultural soil to a final chemical concentration of 50 ng/g soil. Controls received the same final concentration of surfactant in an aqueous addition. Microbial mineralization of the surfactants to ¹⁴CO₂ was measured over a period of 60 days, and the data were fitted to first and mixed ("3/2") order mineralization models. The surfactants were mineralized at the greatest rates when added to the soil as an aqueous solution, or when bound to kaolinite, sand, or illite. They were mineralized to the greatest extents when added in aqueous solution, or when bound to kaolinite or sand. The montmorillonite-bound surfactants were commonly mineralized to lesser extents than the other mineral-bound surfactants. When bound to the humic acids, the mineralization of the chemicals was initially slower than the other forms, but sometimes reached similar extents of conversion to ¹⁴CO₂. In contrast, when bound to the fulvic acids, the chemicals were always mineralized more slowly and to much lower extents than all other forms. The fulvic acid complex rendered two of the surfactants non-bioavailable, as no mineralization was observed. Microbial community characteristics of biomass and physiological status were not correlated with any of the mineralization kinetic estimates. These experiments expand on the findings observed when the fates of the complexed chemicals were studied in a natural woodlot soil. Notably, the mineralization of the chemical-soil constituent complexes showed a wider variability in this soil, compared to the natural soil. Correspondingly, the correlations between the kinetic estimates and desorption coefficients (K_d) showed a wider variability in this soil. This was likely due to the added level of complexity introduced by the sludge amendments. However, the substantially different soil characteristics and mineralogy of this soil did not have as drastic effects on the mineralization kinetics as we expected. These experiments corroborate the conclusions that the environmental form of a chemical has a significant influence upon its eventual microbial metabolism. Furthermore, they demonstrate that interactions with some soil constituents may have little effect on the microbial metabolism of these chemicals, while others (montmorillonite, humic acids and especially fulvic acids) may retard a chemical's bioavailability to the microbial community.

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Multi-Phase Partitioning of Benzene and Toluene in Unsaturated Soils in the Presence of NAPL

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Understanding partitioning behavior in the vadose zone is essential in determining the fate and transport of contaminants. such partitioning may be greatly affected by the presence of a non-aqueous phase in the soil matrix. Contaminants may partition between the soil, non-aqueous liquid, gas, and water phases. Experiments were carried out at varying moisture contents to simulate field conditions and measure the sorption of contaminants into each of these four phases. Dodecane was chosen as the model non-aqueous liquid phase because of its low volatility and affinity for hydrophobic contaminants. Toluene and benzene were chosen as the model contaminants. Experimental results were then compared with predictions made from two-phase equilibrium parameters. Such comparison allowed for the examination of multi-phase partitioning effects over a broad domain of system composition. Results showed that the effects of partitioning between the non-aqueous liquid phase and the soil solid phase were significant at low moisture contents and for high organic matter soils.

Microbial Transformations of Environmental Contaminants during Sulfate Reduction

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Much of the work in our laboratory is focused on the *in-situ* microbial degradation of organic contaminants in soils and subsurface systems under low redox conditions. We are particularly interested in transformations of chloroaromatic compounds, petroleum hydrocarbons, polyaromatic hydrocarbons and metals as mediated by bacterial sulfate reduction. Selected data from several projects will be presented in an effort to demonstrate the emerging role sulfate-reducing microbial communities may play in the natural attenuation of environmental contaminants.

One project involves the sulfate-mediated transformation of two widely used halogenated pesticides, dicamba (3,6-dichloro-2-methoxybenzoic acid) and picloram (4-amino-3,5,6-trichloropyridinecarboxylic acid), both of which are commonly detected in ground water. Microcosms containing soils with a documented history of exposure to dicamba exhibited transformation activity after long lag periods (e.g., 25 weeks). 3,6-dichlorosalicylic acid accumulated as an intermediate. Rates of transformation in the microcosms increased dramatically upon repeated exposure to the compound, although dechlorination was never observed. Picloram transformation was not observed when sulfate was provided as the primary electron acceptor. In a related study, several mono-, di- and tri-chlorinated benzoic acids, phenols and anilines were evaluated for their susceptibility to microbial attack in sulfidogenic sediment microcosms. The three monochlorophenols and 3-chlorobenzoic acid were mineralized during sulfate reduction; all other isomers were apparently recalcitrant under these conditions.

In another study involving coal tar contamination at a former manufactured gas plant (MGP) site, naphthalene was mineralized in sulfate-reducing microcosms which contained subsurface materials from a low redox aquifer. Naphthalene biotransformation under sulfidogenic conditions has previously only been implied from field data. To our knowledge, no other work has yet been published that establishes that naphthalene is transformed under sulfidogenic conditions. Work in our laboratory is currently underway to confirm that naphthalene oxidation is definitively coupled with the reduction of sulfate. Benzene and toluene have also been shown to be mineralized under sulfate-reducing conditions at the site. Benzene degradation during sulfidogenesis has only been documented once, while toluene transformation has been repeatedly reported by several investigators since about 1986. In both cases, however, little or nothing is known of the biochemistry of the reactions or the microorganisms involved.

Recently published work from our laboratory focused on the role sulfate-reducing microbial communities may play in the *in-situ* immobilization of heavy metals and arsenic in sediments and subsurface environments. Our study correlated microbial sulfide formation and arsenic precipitation in actively sulfate-reducing laboratory microcosms which contained arsenic-contaminated sediments. Precipitates were characterized by SEM-EDS. An AsFeS precipitate was identified.

Over the past decade, our understanding of sulfate-reducing bacterial communities has improved immensely. We now know that sulfidogens may utilize a broad range of potential substrates, that they are active in a variety of nonmarine habitats, and may play a role in the remediation of environmental contaminants.

Biodegradation of PAHs in Soil and Sediments

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Manufactured gas plants (MGPs), which operated in most urban areas throughout the United States from the late 1800's to the mid 1900's, produced gas from a combination of coal, coke, and/or oil. During the operation of these plants, process residuals accumulated. As a result, many former MGP sites contain soil that is contaminated with coal tar, a primary source of polycyclic aromatic hydrocarbons (PAHs). Where MGPs were located near bodies of water, sediments are also contaminated. Biodegradation studies of PAHs were conducted with two types of matrix. The matrices used were a harbor sediment with a low concentration of PAHs (*ca.* 1,800 mg total PAHs per kg dry sediment) and an MGP soil with a high concentration of PAHs (*ca.* 30,000 mg total PAHs per kg dry soil). Microbial characterization of each matrix indicated the presence of an indigenous PAH-degrading bacterial population. Aqueous suspensions (1:10) of contaminated soil and sediment were treated in closed, slurry-phase bioreactors to evaluate the degradative ability of the indigenous microflora. The experiments were conducted in batch mode under aerobic conditions, at 23-25°C. The pH and the dissolved oxygen levels were maintained at 7-7.5 and 6-8 mg L⁻¹, respectively. Nitrogen and phosphorus were added to obtain a C:N:P ratio of 100:10:5. Previous experiments with this MGP soil indicated that biodegradation kinetics were enhanced by nutrient amendment. PAHs were extracted from mixed-liquor samples with methylene chloride using sonication and analyzed by GC/MS.

Low-molecular-weight PAHs were degraded first, followed by higher-molecular-weight PAHs. After 40 days, both studies showed a similar pattern of biodegradation for each PAH group. For the sediment, degradation was 95, 97, 77 and 13% for 2, 3, 4, and 5-6 ring PAHs, respectively. For the soil, the degradation was 99, 88, 62, and 30% for the 2, 3, 4 and 5-6 ring PAHs, respectively. For the soil, the degradation was 99, 88, 62, and 30% for the 2, 3, 4 and 5-6 ring PAHs, respectively. By day, *ca.* 80% of the total PAHs were degraded in both matrices. However, a residue of *ca.* 6000 mg kg⁻¹ remained in the sediment. On continued incubation of the soil, up to 70 days, there was no further significant degradation of PAHs. These results show that, although the requisite environmental conditions and microflora are present, other factors such as bioavailability limit the degree of PAH biodegradation attainable.